

FERTILITY OF CONIDIOSPORES OF *PENICILLIUM* SP.
IN THE EXCREMENTS
OF *ENTOMOBRYA PURPURASCENS* (PACKARD)
COLLEMBOLA ENTOMOBRYIDAE

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In our experiment we have compared the fertility of undigested spores and of spores from excrements. Their fertility was of interest in connection with the dispersal of soil fungi in the soil through active spores from excrements.

MATERIAL AND METHODS

We have been culturing the species *Entomobrya purpurascens* for one year in laboratory conditions. We have chosen this species because in previous experiments it had consumed hyphae and spores of our penicillium.

Penicillium sp. had been isolated from woodland mould in plantations of *Picea abies*.

The vessels to keep the springtails in were of plastic material, with a volume of 190 cm³ and gypsum in the bottom. In each vessel about 20 springtails were kept with conidiospores of penicillium mixed into honey. A drop of this mixture, on a piece of impregnated filter-paper, was put onto the gypsum bottom of the vessel. The spores were mixed with honey in order to remain fixed and to preclude any infection of the excrements with nutrient spores.

The fertility was tested in diluted acid potato agar (30 ml H₂O dist + 80 ml acid potato agar of pH 4) by adding diluted agar to the mixture of honey plus spores. After 5 minutes of mixing with the magnetic mixer, a few drops of this mixture were put into Bürker-Türk and Thoma chambers for the counting of erythrocytes. After 22 hours at 25°C, the germinating and the non-germinating spores were counted. The longest germinating hyphae were at the edges of the chambers, and the smallest ones were in the middle. We counted in those parts of the chamber where young hyphae 50-100 μ long, were contained. The volume unit in which we counted, numbered 0,008 mm³ and corresponded to a microscopic visual field of 0,08 mm².

After three days we picked up the excrements. For the first analysis we mixed about 100 excrements with 0,4 ml of agar in a porcelain mortar, and the mixture was cultured in the way described above. For the second analysis the excrements were crushed and mixed with agar on counting slides. We used about 30 excrements.

To avoid an infection of the excrements with outer spores, all the manipulations of collecting the spores and putting them into the counting chambers were performed in an aseptic chamber. So the animals themselves remained the main infection sources, which could bring active spores on their legs and bodies into the vessel.

The excrements were also analyzed on microscopic slides. Besides spores, the excrements

contained remainders of eaten-up springtails and a crushed substance, whose origin we have been unable to determine. We have counted only the intact spores.

The fertility or sterility of undigested spores was determined in three series. Two were carried out at the beginning, and the third one at the end of the experiments. By this analysis the germinative faculty of the spores in the honey was checked, with which we were feeding our animals throughout the experiment.

The fertility of the spores in the excrements was ascertained in two series. Through all series the spores were cultures in chambers for the counting of erythrocytes, i. e. in three such chambers after Bürker-Türk and in two after Thoma.

RESULTS

TABLE I

Spore Fertility in the Nutrient Mixture of Honey plus Spores

	No. of units	Counted spores	Fertile spores	Sterile spores	‰
	0,008 mm ³				
1st count ...	114	1 343	1 328	15	989
2nd count ...	55	543	538	5	991
3rd count ...	63	456	452	4	991

TABLE 2

Spore Fertility in the Excrements

	No. of units	Counted spores	Fertile spores	Sterile spores	‰
	0,008 mm ³				
1st count ...	50	460	1	459	2
2nd count ...	118	1 049	2	1 047	2

As the results have shown, the fertility of *Penicillium* sp. spores in the excrements of the *Entomobrya purpurascens* springtail falls from 990 p. 1000 to 2 p. 1000.

This decrease of fertility has been observed on optically intact spores.

The third analysis of spore fertility in honey has shown the fertility of spores in honey after 22 days (i. e. the end of the experiment) to have remained the same, which means that the fertility of nutrient spores has during the experiments not changed.

RÉSUMÉ

FERTILITÉ DES CONIDIOSPORES DE *PENICILLIUM* SP.
DANS LES EXCRÉMENTS D'*ENTOMOBRYA PURPURASCENS* (PACKARD)
COLLEMBOLA ENTOMOBRYIDAE

Dans notre expérience, nous avons déterminé le pouvoir de germination des spores de *Penicillium* sp. dans des excréments du collembrole *Entomobrya purpurascens*. Nous avons cultivé ces spores dans de l'agar acide de pomme de terre à pH 4. Après 22 heures à 25°C, nous avons compté les spores germées.

La comparaison entre la germination des spores non digérées et celle des spores des excréments a montré que le pouvoir germinatif des spores digérées était tombé de 990 p. 1 000 à 2 p. 1 000.

ACKNOWLEDGEMENTS

I wish to express my gratitude to my mentor Dr Kazimir Tarman for his advice during the work, and to all those having in some way or other contributed to this experiment.

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DISCUSSION

G. ZACHARIAE : Have you any idea of the kind of influence that destroys the spores during the passage through the animal. It is difficult to imagine that mechanical destruction works to such a high percentage, but it a chemical effect possible if the cell walls remain intact ?

S. ČERVEK : It is clear that some kind of chemical influence *i. e.* some enzymatic actions play a role in destruction of germinative ability of digested spores, but we do not know in which way, it is done.

P. BERTHET : In connection with the very interesting paper of Dr. Zinkler that we will hear later, do you think that the reduction of fertility of the spores is due to some mechanical action or to some particular enzymatic actions ?

S. ČERVEK : In reduction of fertility of uncrusted spores from excrements, of course, particular enzymatic actions play a part.

G. J. F. PUGH : Have you fed your *Entomobrya* on spores suspended in a non-nutritive solution ?

S. ČERVEK : We tried to suspend spores in some solutions, but honey showed to be the best because of its conservatory effect on this *Penicillium* spores.